SARTORIUS

Product Guide

# iQue® Fixable Viability Kits

## Product Information

### Presentation, Storage and Stability

The iQue® Fixable Viability Kits contain sufficient quantity of reagents for measurement of cell viability prior to fixation and permeabilization in a 5x96-well format. Included in the kit are: one vial of lyophilized iQue® Fixable Viability (either V/Blue, B/Green, or R/Red) Dye, one vial of anhydrous DMSO, and a USB Flash Drive containing analysis templates.

Upon receipt, the iQue® Fixable Viability Kits should be stored at -20°C. The kits are stable to the expiration date stated on the vial label. Once solubilized in DMSO, iQue® Fixable Viability Dye stock solution can be aliquoted and stored at -20°C for up to three months and should be protected from light and moisture. Freeze-thaw cycling should be avoided.

| Product Name | Detection Channel | Cat No | Format |
|---|---|---|---|
| iQue® Fixable Viability (V/Blue) Kit | VL-1 | BA-97116 | 5x96-well |
| iQue® Fixable Viability (B/Green) Kit | BL-1 | BA-97119 | 5x96-well |
| iQue® Fixable Viability (R/Red) Kit | RL-1 | BA-97122 | 5x96-well |

**Table 1.** Product Information
Note: A kit product guide and a USB key with assay templates are also included in the kit package.

| Kit Components | 5x96-well | Storage | Stability |
|---|---|---|---|
| iQue® Fixable Viability (either V/Blue, B/Green, or R/Red) Dye | 1 vial (lyophilized) | -20°C | Refer to vial label for expiration date. Dye stock solution is stable for up to 3 months after reconstitution. |
| DMSO | 1 vial (150 µL) | -20°C | |

**Table 2.** Kit Components and Storage
Note: The safety data sheet (SDS) can be found on our website www.sartorius.com.

## Background

The iQue® Fixable Viability Dyes are validated to quantify live and dead cells using flow cytometry post fixation, permeabilization and intracellular staining of markers (immunophenotyping) and cytokines without any loss of intensity. Dyes are cell membrane-impermeant that covalently label cell surface and intracellular amines, resulting in dim surface staining of live cells and highly fluorescent staining of cells with compromised membranes. The staining is stable, and the fluorescence is preserved following fixation and permeabilization. The iQue® Fixable Viability Kits are optimized to run on the iQue® platform, with BR and VBR configurations. Fixable Viability Kits are available in three different colors for multi-color flow panel needs. Pre-set templates are included for gating strategy and analysis for the ease of use.

## Recommended Use

The iQue® Fixable Viability Kits are used to label viable from non-viable mammalian cells prior to downstream fixation and permeabilization, which is required for intracellular cell staining. The kits are compatible with formaldehyde and methanol-based fixatives, and have been validated in Jurkat, Raji, Ramos, THP-1, PBMC, and mouse splenocytes. The kits have not been optimized for use in adherent cell lines. This product is designed for research purposes only.

## Workflow

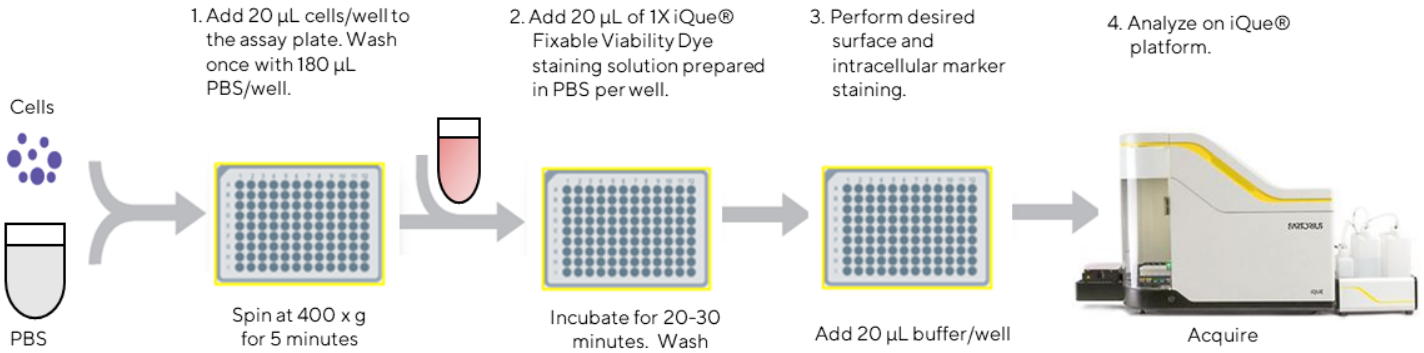


**Figure 1**: iQue® Fixable Viability Kit workflow.

# Protocol and Procedure

## 1. Prepare 1X Dye Staining Solution

1.1 Allow iQue® Fixable Viability Dye and DMSO to reach room temperature (RT) prior to assay.

1.2 Reconstitute one vial of lyophilized dye by adding 100 µL DMSO to the dye vial and mix until fully dissolved. This is the 200X dye stock solution.

Note: The 200X dye DMSO stock solution can be aliquoted and stored at -20°C for up to three months, should be protected from light and moisture.

1.3 Prepare 1X Dye Staining Solution by adding 10 µL 200X dye DMSO stock solution per 2 mL phosphate-buffered saline (PBS, without Ca2+ or Mg2+, pH 7.4) free of azide, proteins, or other amine-containing compounds.

1.4 Use the 1X Dye Staining Solution within one hour after preparation.

## 2. Stain Cells with Fixable Viability Dye

2.1 Count cells and adjust cell number to 1-5 x $10^6$ cells/mL with culture medium.

Note: To acquire sufficient amount of cells for proper data analysis, it is recommended to use at least 2x $10^6$ cells/mL if the dye-stained cells are fixed and permeabilized for subsequent intracellular staining.

2.2 Add 20 µL cells to each sample well of a 96-well assay plate.

2.3 Wash cells once by adding 180 µL protein free PBS to each well, and centrifuge at 400 x g for 5 minutes. Aspirate supernatant.

2.4 Shake the plate (3000 RPM, 60 seconds) to resuspend cells in residual liquid.

2.5 Add 20 µL iQue® Fixable Viability Dye 1X Staining Solution prepared in Step 1.3 to each well.

2.6 Give the assay plate a quick spin (400 x g, 5 seconds) and brief shake (2000 RPM, 20 seconds) to ensure that all samples are thoroughly mixed at the well bottom.

2.7 Incubate samples for 20-30 minutes, RT, and protect from light.

2.8 Add 180 µL/well PBS+0.5% BSA, pH7.2 to the stained cells and centrifuge at 400 x g for 5 minutes. Aspirate supernatant.

2.9 Shake the plate (3000 RPM, 60 seconds). Continue the antibody staining procedure described in part 3 or proceed to step 3.5 for cell viability analysis without fixation.

## 3. Stain Cell Surface and Intracellular Markers

3.1 Solutions used in this protocol

3.1.1 Cell Staining Buffer: PBS (without Ca2+ or Mg2+), 0.5% BSA, pH 7.2-7.4

3.1.2 Fixation/Permeabilization Buffer: 3.7% formaldehyde, 0.1% saponin in PBS (without Ca2+ or Mg2+, pH 7.4)

3.1.3 Permeabilization/Wash Buffer: PBS (without Ca2+ or Mg2+), 0.5% BSA, 0.1% saponin, pH 7.2-7.4

3.2 Stain cell surface markers

Notes:

a. It is recommended to stain surface markers prior to fixation/permeabilization, as some antibodies recognizing cell surface markers may not bind to fixed/denatured antigens.

b. Remember to include appropriate controls for setting compensation and proper data analysis.

3.2.1 Add 20 µL/well cell staining buffer containing pre-determined optimal concentration of fluorescently labeled surface marker antibodies to the desired sample wells.

3.2.2 Give the assay plate a quick spin (400 x g, 5 seconds) and brief shake (2000 RPM, 20 seconds).

3.2.3 Incubate the samples at RT for 30min, protect from light.

3.2.4 Add 180 µL/well cell staining buffer to each well. Centrifuge at 400 x g for 5min. Aspirate supernatant.

3.2.5 Shake the plate (3000 RPM, 60 seconds). Continue step 3.3 for cell fixation and permeabilization or proceed to step 3.5.

3.3 Fix and permeabilize cells.

3.3.1 Add 20 µL/well of Fixation/Permeabilization Buffer to cells.

3.3.2 Give the assay plate a quick spin (400 x g, 5 seconds) and brief shake (2000 RPM, 20 seconds).

3.3.3 Incubate the samples at RT for 15 minutes, protect from light.

3.3.4 Add 180 µL permeabilization/wash buffer to each well. Centrifuge at 400 x g for 5min. Aspirate the supernatant.

3.3.5 Add 200 µL permeabilization/wash buffer to each well. Centrifuge at 400 x g for 5min. Aspirate the supernatant for thorough removal of residual fixative.

Note: Alternatively, cells can be fixed and permeabilized with 20 µL/well of pre-cooled methanol for 20 minutes at -20°C, wash twice following steps 3.3.4 and 3.3.5 with cell staining buffer.

3.3.6 Shake the plate (3000 RPM, 60 seconds).

3.4 Stain for intracellular markers

3.4.1 Add 20 µL/well of fluorescently labeled antibodies for specific intracellular targets that are diluted in permeabilization/wash buffer to the desired wells.

3.4.2 Give the assay plate a quick spin (400 x g, 5 seconds) and brief shake (2000 RPM, 20 seconds).

3.4.3 Incubate the sample for 30 min at RT, protected from light.

3.4.4 Add 180 µL permeabilization/wash buffer to each well. Centrifuge at 400 x g for 5 min. Aspirate the supernatant. Shake the plate (3000 RPM, 60 seconds)

Note: If the cells are fixed and permeabilized with methanol, use cell staining buffer for steps 3.4.1 and 3.4.4.

3.5 Add 20 µL cell staining buffer to each well. Quick spin (400 x g, 5 seconds) the plate, and run the samples on iQue®.

## 4. Plate Acquisition and Data Analysis

4.1 Launch iQue Forecyt® Software.

4.2 Import the provided experiment template (included on USB key in the kit package).

4.3 Create a New Experiment using the provided template.

4.4 In the Design section, assign wells to Sample or desired well type.

4.5 In the Protocol section, adjust Sample Order if plate layout requires (vertical instead of horizonal), adjust sip time to ensure appropriate amounts of cells are acquired.

4.6 Click "Run" on the Controller window to acquire the plate.

4.7 Use the included template to gate viable and non-viable cell populations for subsequent analysis.

## Example Data

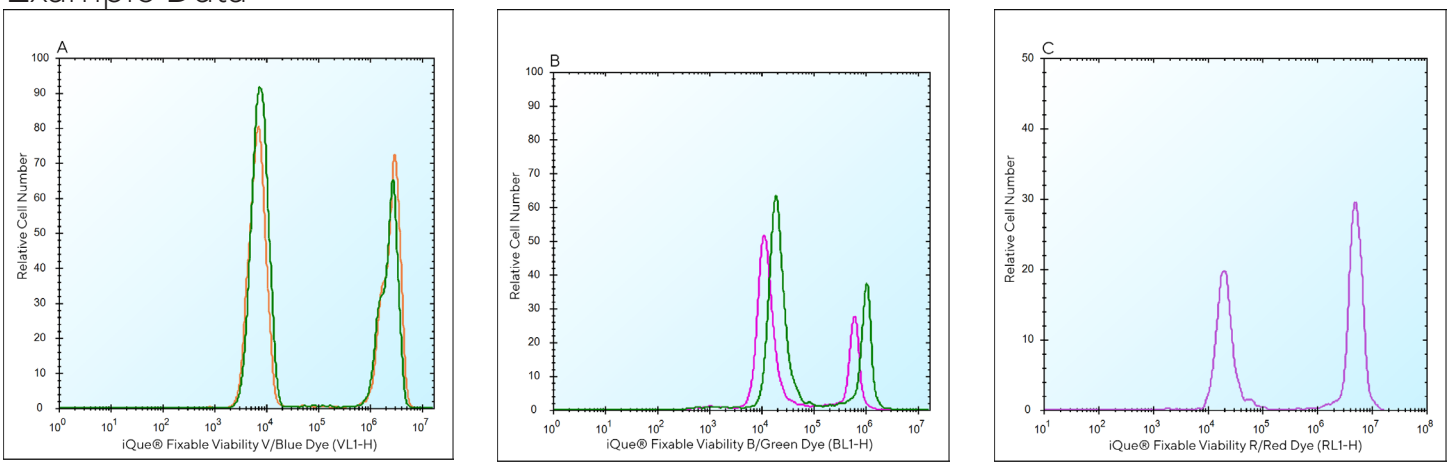


**Figure 2:** Representative histograms of dead staining with iQue® Fixable Viability Dyes. A). Two samples of live and heat-treated (65°C for 10 minutes) Jurkat cell mixtures were stained with iQue® Fixable Viability (V/Blue) Dye. One sample was fixed with 3.7% formaldehyde (orange line), the other sample was not fixed (green line). The samples were analyzed in VL1 channel of iQue®. B). Mouse splenocytes were stained with iQue® Fixable Viability (B/Green) Dye (pink line) and fixable green viability dye from a vendor (green line) followed by fixation/permeabilization with 3.7% formaldehyde, 0.1% saponin in PBS. Similar dead cell staining patterns were obtained when analyzed in BL1 channel of iQue®. C). A mixture of live and heat-treated (65°C for 10 minutes) Jurkat cells were stained with iQue® Fixable Viability (R/Red) Dye, fixed with 100% methanol, and analyzed in RL1 channel of iQue®.

# iQue® Fixable Viability Kits Quick Guide

## Reagent Preparation

- [ ] Allow included kit reagents to reach room temperature (RT) prior to assay.

⇩

- [ ] Reconstitute lyophilized dye with 100 µL DMSO to make 200X Stock.

⇩

- [ ] Prepare 1X dye staining solution by diluting dye DMSO stock 200-folds in PBS. Use within an hour.

*Start time* _____ *Use time* _____

## Assay Protocol

- [ ] Add **20 µL/well** Cell Samples to the assay plate, **180 µL/well** PBS.
Spin at 400 x g, 5 minutes. Aspirate supernatant, shake 3000 RPM, 60 seconds.

⇩

- [ ] Add **20 µL/well** 1X Dye Staining Solution.
Quick Spin|Brief Shake*
**Incubate RT 20–30 min, Dark**

*Start time* _____ ⇩ *Stop time* _____

- [ ] Add **180 µL/well** PBS+0.5% BSA. Spin at 400 x g, 5 minutes. Aspirate supernatant, shake 3000 RPM, 60 seconds.

⇩

- [ ] Perform desired cell surface marker staining, fixation/permeabilization and intracellular staining.
Record each procedure in the notes below if needed.

⇩

- [ ] Add 20 µL/well PBS+0.5% BSA. Quick Spin. Acquire Data.

*Quick Spin |Brief Shake =400 x g, 5 sec. | 2000 rpm, 20 sec.

**Notes:**

# Sales and Service Contacts

## For further information, visit www.sartorius.com

**Essen BioScience, A Sartorius Company**

www.sartorius.com/ique



Publication No. #17218 Rev A.

**North America**
Essen BioScience Inc.
300 West Morgan Road
Ann Arbor, Michigan, 48108
Telephone +1 734 769 1600
E-Mail: AskAScientist@sartorius.com
Online Store: shop.intellicyt.com

**Europe**
Essen BioScience Ltd.
Units 2 & 3 The Quadrant
Newark Close
Royston Hertfordshire
SG8 5HL
United Kingdom
Telephone +44 (0) 1763 227400
E-Mail: euorders.UK03@sartorius.com

**APAC**
Essen BioScience K.K.
4th floor Daiwa Shinagawa North Bldg.
1-8-11 Kita-Shinagawa
Shinagawa-ku, Tokyo
140-0001
Japan
Telephone: +81 3 6478 5202
E-Mail: orders.US07@sartorius.com